

## RFP-Booster for Immunofluorescence Detection of RFP-Fusion Proteins

For the immunofluorescence detection of RFP-fusion proteins in fixed cells.

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**1. Product** The RFP-Booster Alexa Fluor® 647 is an anti-RFP Nanobody coupled to Alexa Fluor® 647.

**2. Introduction** Red fluorescent proteins (RFP) and its variants are widely used to study protein localization and dynamics in cells. However, photo-stability and quantum efficiency of RFP are often not sufficient for e.g. super-resolution microscopy (such as 3D-SIM or dSTORM) and for fixed cell samples. In addition, many cell biological methods such as BrdU-staining, EdU-Click-iT™ treatment or fluorescent *in situ* hybridization result in disruption of the RFP signal. The RFP-Booster reactivates, enhances, and stabilizes the RFP-signal.

*Note: This product is an improved version of product rba647n.*

### 3. Properties

Product size	rb2AF647-10: 10 µL rb2AF647-50: 50 µL
Format	Alpaca single domain antibody, Nanobody or V <sub>H</sub> H; monovalent
Target/ Specificity	RFP and RFP variants. See <a href="http://www.chromotek.com">www.chromotek.com</a> for a list of recognized RFP variants.
Conjugate	Site-directed conjugation to Alexa Fluor 647
Excitation/ Emission	Excitation max: 650 nm, Emission max: 665 nm
DOL	2 fluorophores per Nanobody
Purity	Recombinantly expressed and purified
Form	Buffered aqueous solution
Storage buffer	10 mM HEPES pH 7.0, 500 mM NaCl, 5 mM EDTA, Preservative: 0.09% Sodium azide, Safety datasheet (SDS): <b>Sodium azide SDS</b>
Concentration	0.5 g/L
Stability and storage	Shipped at ambient temperature. Store at -20°C/-4°F. Avoid freeze-thaw cycles. Aliquot upon arrival. Protect from light. Stable for 6 months.

### 4. Protocol

- Fixation:** Fix cells seeded on coverslips in 3.7% formaldehyde in PBS for 10 min at room temperature.  
*Note: Always prepare a fresh formaldehyde dilution.*  
*Note: Alternatively, use methanol for fixation: Apply ice-cold 100% methanol to cells for 3 min, wash as in step 2 and proceed directly with step 5 of this protocol.*
- Wash samples three times with PBS (Phosphate Buffered Saline). Do not store fixed cells.
- Permeabilization:** Add PBS containing 0.5% Triton X-100 to samples and incubate for 5 min at room temperature.
- Wash samples twice with PBS.
- Blocking:** Add 4% BSA in PBS to samples and incubate for 10 min at room temperature.
- RFP-Booster incubation:** Dilute RFP-Booster 1:500 – 1:1,000 in blocking buffer and incubate for 1 h at room temperature. Optimal dilution is application-dependent and should be determined.  
*Note: For multiplexing protocols, you can combine RFP-Booster with any other antibody.*
- Wash samples three times for 5-10 min in PBS.
- If required, counter stain with DNA fluorescent dyes, e.g. DAPI in PBS.

9. **Mounting:** Rinse sample shortly in water to prevent formation of salt crystals. Mount in VectaShield (Vector Labs) or other mounting media with anti-fading agents and seal mounted coverslips with clear nail polish.

**Suggested buffer composition**

Buffer	Composition
Blocking buffer	4% BSA (w/v) in PBS
Fixation buffer	3.7% formaldehyde in PBS
Permeabilization buffer	PBS; 0.5% Triton X-100
Wash buffer	PBS

**5. Support/  
Troubleshooting**

Please refer to our FAQ section at [www.chromotek.com](http://www.chromotek.com) for or contact [support@chromotek.com](mailto:support@chromotek.com)

*Only for research applications, not for diagnostic or therapeutic use.*

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